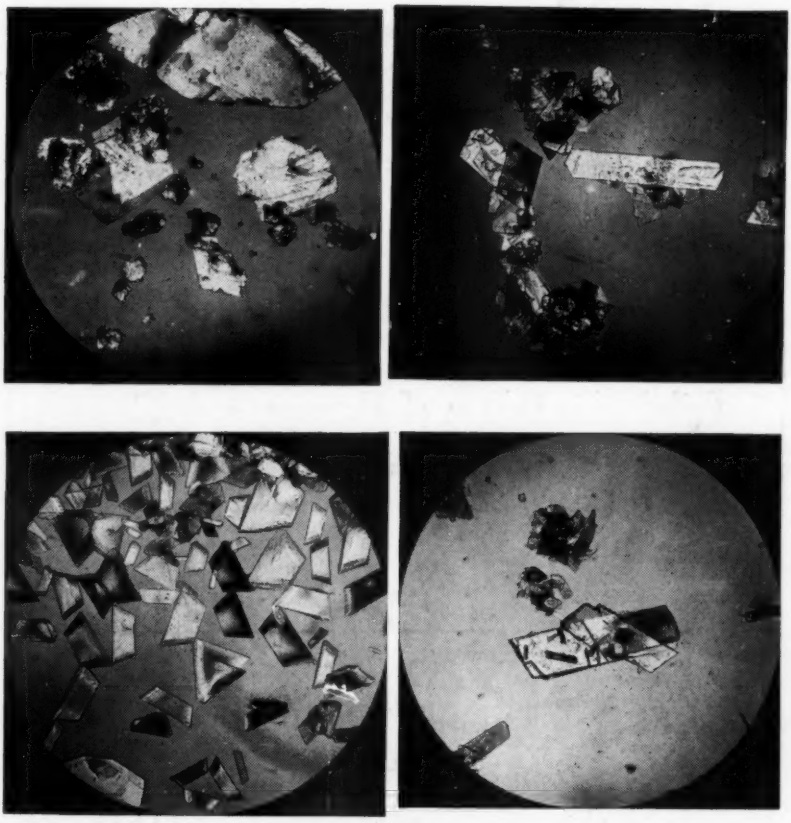
ODICAL

American Journal of Pharmacy

NOV 29 1946

AND THE SCIENCES
SUPPORTING PUBLIC HEALTH



MICROPHOTOGRAPHS OF AMINO ACID CRYSTALS

See review article on Amino Acid Therapy, p. 320.

Since 1825 *September 1946*

Education in

Pharmacy—Chemistry

Bacteriology—Biology

opens the way to many opportunities and their own future rewards. This College offers B. Sc. degree courses in all four of these sciences. Also selected graduate studies. Write for details and a new catalog for the 1947 and the 1948 terms. Inquiries welcome from veterans who are eligible for education under the Veterans Administration.

Philadelphia College of Pharmacy and Science

Founded 1821

43d St., Woodland and Kingsessing Aves.

Philadelphia 4, Pa.

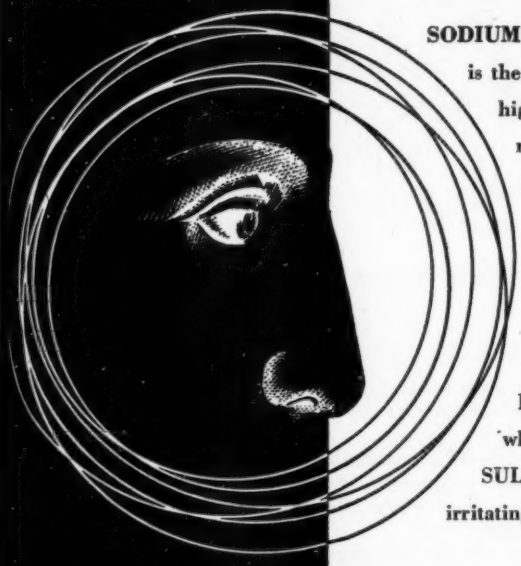
American Journal of Pharmacy

Published monthly by the Philadelphia College of Pharmacy and Science
43d Street, Kingsessing and Woodland Avenues, Philadelphia 4, Pa.

Annual Subscription, $3.00 — Foreign Postage, 25 Cents Extra
Single Numbers, 30 Cents — Back Numbers, 50 Cents

Entered as Second-Class Matter March 27, 1937, at the Post Office at Philadelphia, Pa.
Under Act of March 3, 1879

Schering INTRODUCES A NEW Rx



SODIUM SULFACETIMIDE SOLUTION 30% is the new sulfonamide solution found to be highly and rapidly effective in the treatment and prophylaxis of most common eye infections. Its superiority over other sulfonamides arises from the fact that it is the only one among all sulfonamides that is soluble to a concentration as high as 30 per cent at physiologic pH. It is, therefore, more bacteriostatic locally, especially for eyeball tissues which it penetrates speedily. SODIUM SULFACETIMIDE SOLUTION 30% is non-irritating, and mildly alkaline (pH 7.4).

SODIUM SULFACETIMIDE SOLUTION 30% (*Sodium* SULAMYD)

is available in 15 cc. amber, eyedropper bottles. Refrigeration is not required. Ample stocks are available now.

Trade-Mark SULAMYD—Reg. U.S. Pat. Off.

SCHERING

Schering CORPORATION • BLOOMFIELD, N. J.

IN CANADA, SCHERING CORPORATION LIMITED, MONTREAL

to combat

mental depression in the menopause

"... because the involutional period is fraught with sadness the different forms of mental disorder of this age may be highly colored with mental depression."*

Severe menopausal depression, marked by apathy and psychomotor retardation, is frequently progressive. Hence, if not promptly and effectively treated, it may seriously impair the patient's normal capacity for useful living.

In such cases, Benzedrine Sulfate helps to overcome the depression, to restore optimism and to reawaken the savor and zest of life. Needless to say, Benzedrine Sulfate is not indicated in the casual case of low spirits, as distinguished from true prolonged mental depression.

*Hinsie, Leland E.: The Person in the Body, an Introduction to Psychosomatic Medicine, New York, W. W. Norton & Co., 1945, p. 223.

b

Tablets and Elixir

benzedrine sulfate

(racemic amphetamine sulfate, S.K.F.)

Smith, Kline & French Laboratories, *Philadelphia, Pa.*

AMERICAN JOURNAL OF PHARMACY

AND THE SCIENCES SUPPORTING PUBLIC HEALTH

Since 1825

LINWOOD F. TICE, Ph. G., B. Sc., M. Sc., Editor
Austin A. Dodge, Ph. C., B. Sc., Ph. D., Assistant to the Editor
John E. Kramer, B. Sc., Business Manager

COMMITTEE ON PUBLICATION

E. Fullerton Cook, P. D., Ph. M., M. S., Chairman
Mitchell Bernstein, P. D., M. D., F. A. C. P.
Louis Gershenfeld, P. D., Ph. M., D. Sc.
Marin S. Dunn, A. M., Ph. D.
John K. Thum, Ph. G., Ph. M.
J. W. Sturmer, Phar. D., Ph. M., D. Sc., Secretary
Ivor Griffith, P. D., Ph. M., D. Sc., F. R. S. A., ex officio

Vol. 118. SEPTEMBER, 1946 No. 9

CONTENTS

Editorial:

Truth vs. Fiction 300

Articles:

Amino Acids, Proteins, and Protein Hydrolysates. By L. F. Tice 302

A Neutralizing Medium for Evaluating the Germicidal Potency of the Quaternary Ammonium Salts. By R. Quisno, I. W. Gibby and M. J. Foter 320

A New Procedure in the Determination of Alcohol in Galenical Preparations by the Customary Distillation Method II. By S. Waldbott 324

Selected Abstracts 328

EDITORIAL

TRUTH vs. FICTION

THIS issue features a review article on amino acids, proteins and protein hydrolysates. Amino acid therapy has aroused no end of interest on the part of the medical and pharmaceutical professions. Physicians are impressed with the frequent and almost miraculous effects of this therapy in shortening convalescence, promoting the healing of ulcers etc., and pharmacists are troubled by their inability to match supply with demand.

Like many another instance hindsight is indeed more simple than foresight and it seems strange that the use of hydrolysates in therapy was not thought of long ago. We well remember in our student days being taught the preparation of predigested milk, using compound pancreatin powder and how this product, a forerunner of the modern hydrolysate, was considered almost obsolete and of doubtful value. We could name many other instances wherein our teachers, and later we ourselves as teachers, were wrong; aqueous extracts of ergot were described as inactive and viburnum as completely inert. The cautious and wise teacher today takes care in explaining that truth is a difficult thing to establish and that our understanding of what constitutes truth varies constantly. As one of our colleagues has so aptly put it "Truth is what I believe", a definition which becomes more understandable the more one reflects on the subject. It is certain that truth concerning a given matter is not considered in an identical manner by all people and who is there capable of acting as a final arbiter on what constitutes truth? History has proven the majority opinion to be wrong on countless occasions, educators wrong, the state and even the church frequently wrong. The best that anyone can hope to do is to evaluate all the evidence to date and submit an opinion constituting what is believed to be truth based on this evidence and with the reservation that future discoveries or a change in man's way of thinking may reverse the situation and prove today's truth total fiction.

We are too often inclined to laugh at some of the patent errors of the past made by men of science, not realizing that these men exercised their best judgment, based on the existing facts at hand. If we ourselves only knew it, many of the things we believe as fundamental facts are no more true than the conclusion reached by men of science several decades past "that it was impossible for heavier-than-air craft to fly". Future generations will doubtlessly laugh at our established truths which many a youngster now in the cradle will some day disprove. We shall then stand up no better than the men whose pronouncements we now ridicule.

It is a fine thing to be young with a fresh college degree, for then there are so many things about which we are so certain. As we grow older and more experienced we are certain about fewer and fewer things. It is for this reason that young men act and older men deliberate and also for this same reason that every council needs both young and old if its actions are to be both progressive and still wise.

Concerning the amino acids all this has but scant relevancy but does it not seem likely that other advances in therapy may be just before our nose, so to speak, awaiting someone with enough imagination to develop them? After development many will say as we have said before "Why didn't I think of that?" or "Why, I had observed that phenomenon but thought it insignificant." Indeed there are yet many new discoveries and new uses of old facts which still challenge those who seek them.

L. F. Tice.

AMINO ACIDS, PROTEINS, AND PROTEIN HYDROLYSATES

By L. F. Tice

Introduction

THE word *protein* was coined by the Dutch chemist, Mulder, from the Greek meaning "of first importance". Although this was over one hundred years ago, it is only during the last few decades that the primary importance of proteins in all life processes has been fully realized. Proteins are essential for every living cell, whether plant or animal, but since animal tissues are built up almost entirely of protein the animal is more dependent on protein than plants which are composed principally of the carbohydrate cellulose. Furthermore, the animal is completely dependent upon other animals or plants consumed as food for protein while plants can manufacture their essential protein from inorganic nitrogen compounds in the soil and even, as in certain legumes, from atmospheric nitrogen.

In the case of animals proteins may be said to be the structural material from which the body is made. It is for this reason that animal tissues when they undergo decomposition or are burned give off a totally different odor than decaying or burning plant tissues.

The proteins themselves are made up of large numbers of amino acid molecules combined in highly specific but complex patterns to form the thousands of different proteins found throughout nature. The number of possible combinations with these amino acids runs into astronomical figures so it is easy to see why each protein differs from all others in its molecular pattern. Amino acids, since they represent the fundamental units from which proteins are made, are often termed the "building blocks of the protein".

In the digestion of protein these large molecules are broken up by hydrolysis, under the influence of the proteolytic digestive enzymes pepsin and pancreatic trypsin, into small segments including amino acids themselves. In this form they are assimilated into the blood stream and then reconstituted into the specific proteins needed by the body. If on the other hand the foreign protein is injected directly into the blood stream a violent reaction known as protein shock is produced which may be fatal.

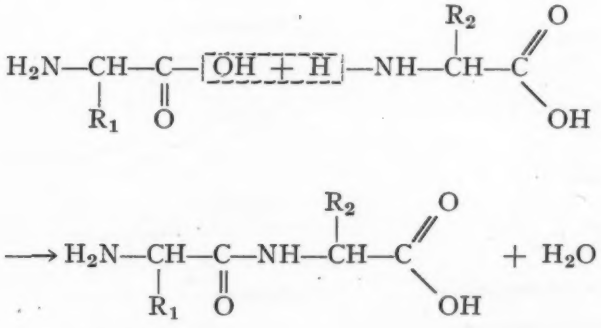
Chemistry of the Amino Acids

Braconnot in 1820 was the first to isolate a crystalline amino acid from a protein. He hydrolyzed gelatin and obtained crystals of glycine which he called "sucre de gelatine" due to the sweet taste of the crystals.

The number of different amino acids that have been isolated as hydrolysis products of proteins is about forty. Chemically, they are organic acids possessing an amino (NH_2) or an imino (NH) group in place of one of the hydrogens attached to the alpha carbon atom. Thus their general formula is $R.CH(NH_2)COOH$. The names and structural formulas of the most important of these are shown in Table 1.

With the exception of glycine all of the amino acids are optically active since they contain one or more asymmetric carbon atoms. The synthesis of amino acids in the laboratory gives rise to racemic mixtures (dl) which are equimolecular mixtures of the dextro and laevo isomers. Although these isomers are chemically identical, they have marked differences in their biological properties which will be discussed later.

The manner by which the various molecules of amino acids are linked together to form proteins was explained by the classic research of Emil Fischer. It was he who demonstrated the coupling of amino acids into peptides shown in the following scheme.

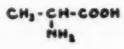


Fischer was able to build up long chains of such coupled amino acids into compounds which possessed properties analgous to those of proteins. The so-called peptide linkage wherein the carboxyl

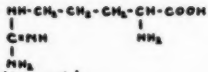
TABLE 1

THE AMINO ACIDS

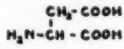
Alanine



Arginine

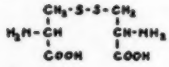


Aspartic acid

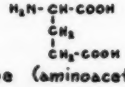


Cysteine

Cystine

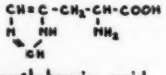


Glutamic acid

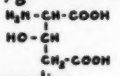


Glycine (aminoacetic acid)

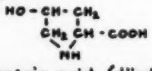
Histidine



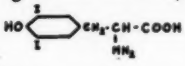
Hydroxyglutamic acid



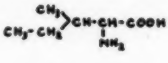
Hydroxyproline



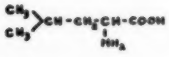
Iodogorgoic acid (diiodotyrosine)



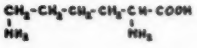
Isoleucine



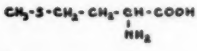
Leucine



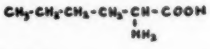
Lysine



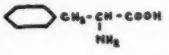
Methionine



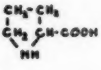
Norleucine



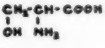
Phenylalanine



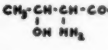
Proline



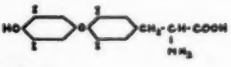
Serine



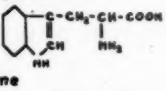
Threonine



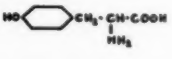
Thyroxin



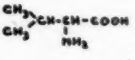
Tryptophane



Tyrosine



Valine



group of one amino acid is linked to the amino group of another is now believed to be the arrangement by which amino acids are built up into protein. It is at these linkages that hydrolysis splits the proteins, ultimately into their constituent amino acids. True proteins have never been synthesized since their molecular weights are very great (e. g. serum globulin 175,000) which means that hundreds of amino acid molecules are present and arranged in an exact pattern. The number of possible patterns for a given amino acid content runs into millions. It is for this reason that two proteins might have almost identical molecular weights and yield the same amino acids but still be totally different in nature and behavior.

The classification of proteins is rather arbitrary and it is based principally upon their physical properties. Thus we have briefly the following types:

1. Simple Proteins—complex combinations of amino acids
    a. Albumins
    b. Globulins
    c. Prolamins
    d. Glutelins
    e. Scleroproteins
        1. Collagens
        2. Elastins
        3. Keratins
    f. Histones
    g. Protamines

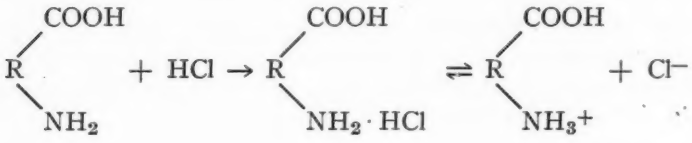
2. Conjugated Proteins—complex combinations of amino acids combined with prosthetic groups of a non-protein nature.
    a. Nucleoproteins
    b. Glycoproteins
    c. Phosphoproteins
    d. Chromoproteins
    e. Metalloproteins
    f. Lipoproteins

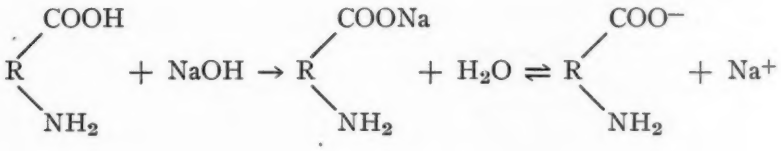
3. Derived Proteins—proteins partially hydrolyzed.

4. Proteins of Endocrine Origin—e. g., insulin, prolactin, etc.

It is not the purpose of this paper to discuss the many differences in behavior between these various types of protein.

Insofar as the amino acids are concerned, they have certain properties which are of primary importance in understanding their behavior. Like the proteins they are ampholytes, i. e., they act as both acids and bases. The old concept of this behavior considers the amino acid to react with either acid or alkali to form a salt as follows:





The modern explanation uses the Zwitterion theory which postulates the compound as a dipolar ion thus:

$$+NH_3.R.COO-$$

The addition of an acid represses the ionization of the carboxyl group resulting in a positively charged ion (cation).

$$+NH_3.R.COOH$$

The addition of a base represses the ionization of the NH_3 group giving a negative ion (anion).

$$NH_2.R.COO-$$

In either of these mechanisms the result is the same. That pH at which the cations and anions equal one another is known as the isoelectric point. At this pH a protein or amino acid has certain maxima and minima properties. For example, solubility is at a minimum

so that the isolation of a given protein or amino acid can best be accomplished at this point. Use is made of this fact in the fractionation of blood proteins and in the separation of pure amino acids.

Protein Metabolism

Mention has been made of the process of digestion whereby the dietary proteins are broken up into peptids and amino acids under the influence of pepsin, trypsin and other enzymes of *succus entericus.* These protein building blocks, as they might be called, are then absorbed through the intestinal mucosa into the portal circulation. Not all proteins are digested with the same ease in the gastrointestinal tract and undigested protein is eliminated in the feces. The so-called "coefficient of digestibility" is determined by an estimation of ingested and fecal nitrogen. These coefficients may differ considerably as shown by the following figures: meat 97 per cent, cereals 85 per cent, and legumes 78 per cent. The peptids and amino acids assimilated from the intestine are carried by the portal vein to the liver. Here blood proteins are formed from part of the supply, the remainder continuing in the systemic circulation by which they are carried to the various tissues of the body for the construction of many essential proteins, hormones, etc. Some of the amino acids entering the liver via the portal vein and others resulting from the constant "wear and tear" of body tissues are deaminated in the liver forming urea $CO(NH_2)_2$ which is excreted by the kidneys. The deamination of certain of the amino acids leads to carbohydrate-like substances which can then be utilized for the energy requirements of the body or converted to glycogen or fat and stored for future use.

It is estimated that in man alone over 1600 different proteins are present in order to carry on the many functions of the body. There is no storage protein or reserve protein in the body as there is storage fat and carbohydrate (glycogen). The body therefore must have a fairly constant supply of protein food or else it is forced to sacrifice certain tissues of the body for the maintenance of essential functions.

Growth, pregnancy, and lactation increase the protein requirements of the individual. The daily intake recommended is as follows:

Man (body weight of 70 Kg.), moderately active, 70 Gm.; Woman (56 Kg.), moderately active, 60 Gm.; in latter half of pregnancy, 85 Gm., and during lactation, 100 Gm.; children under 1 yr., 3-4 Gm. per Kg., 1-3 yrs., 40 Gm., 4-6 yrs., 50 Gm., 7-9 yrs., 60 Gm., 10-12 yrs., 70 Gm.; Girls, 13-15 yrs., 80 Gm., 16-20 yrs., 75 Gm. and Boys, 13-15 yrs., 85 Gm., 16-20 yrs., 100 Gm.

In determining the protein content of foods a nitrogen determination is performed using the Kjeldahl method and then multiplying the % N by a factor of 6.25. This factor is based on the observation that many proteins contain about 16 per cent nitrogen.

Many years ago it was observed that neither man nor other animals could survive when certain single proteins constituted their only protein food, while other single proteins such as casein were able to maintain the animal in complete health. This led to the designations complete and incomplete proteins.

Nitrogen Balance and Essential Amino Acids

When the ingested nitrogen in the diet equals that excreted, as during growth, the animal is said to be in positive balance. A negative balance is that condition wherein the excreted nitrogen exceeds that ingested as during starvation, illness, etc.

As early as 1906 Willcock and Hopkins, and later, Osborne and Mendel showed that rats on synthetic diets containing a single incomplete protein could not maintain nitrogen balance. The addition of certain amino acids to this diet enabled the animals to achieve nitrogen balance and grow. It was thus established that certain amino acids which the body cannot synthesize are required for normal growth. These amino acids are called the "essential amino acids".

Rose and his co-workers at the University of Illinois have carried out some very fundamental researches investigating the identity of these essential amino acids. These workers employed synthetic diets, all of the nitrogen of which was furnished by mixtures of crystalline amino acids. At first they found that a mixture of all of the then known amino acids failed to produce nitrogen balance in the rat. This led to the discovery of a previously unknown amino acid, threonine, which when added to the mixture provided positive nitrogen balance. By altering the components of the mixture Rose finally was able to classify the amino acids with respect to their necessity for the growth of the rat. His table is presented in Table 2.

TABLE 2

CLASSIFICATION OF AMINO ACIDS WITH RESPECT TO THEIR GROWTH EFFECTS IN RATS

Essential	*Non-Essential*
Arginine	Alanine
Histidine	Aspartic Acid
Isoleucine	Citrulline
Leucine	Cystine
Lysine	Glutamic Acid
Methionine	Glycine
Phenylalanine	Hydroxyglutamic Acid
Threonine	Hydroxyproline
Tryptophane	Norleucine
Valine	Proline
	Serine
	Tyrosine

The rat is able to do without those listed as non-essential not because they are not needed but because they can be synthesized by metabolic processes providing the essential ones are given in adequate amounts. One authority has described the non-essential character of certain of the amino acids in a rather droll way as follows: "The non-essential amino acids are so essential that the body has learned how to synthesize them".

Although these ten essential amino acids hold true for the rat other animals do not, in every case, conform to the same requirements. For example, glycine is essential for the chick but not for the rat. Human beings require only *eight* essential amino acids, the same as those listed for the rat in Table 2 minus arginine and histidine.

In examining the amino acid content of various proteins the observation has been made that cereal and vegetable proteins are relatively poor in lysine. This is probably the reason for their lower nutritive value as compared with proteins of animal origin. The completeness of milk as a protein food is seen in the amino acid content of both casein and lactalbumin.

Protein Deficiency

A deficiency of protein with its resulting sequelae can be caused by any one of three basic conditions, namely,

(a) insufficient protein intake
(b) improper utilization
(c) excessive loss, excretion or catabolism

In the first instance the protein intake may be insufficient in quantity or it may be of poor biological value or quality. Protein cannot be stored in the body as in the case of fat and carbohydrate. Fat depots throughout the body are common and even normal and carbohydrate is stored in the liver as glycogen. With proteins there is a constant turnover or a dynamic equilibrium with no reserve available except body protein such as tissue protein or serum albumin which can be utilized in periods of need.

Certain proteins are poor in quality either because they are digested with great difficulty or because they do not contain all of the essential amino acids. Thus corn and peas, although rich in protein, are inferior to meat and eggs. It is for this reason that a strictly vegetarian diet excluding milk is inferior to one containing animal protein. Table 3 lists the per cent of essential amino acids in various protein foods.

TABLE 3

PER CENT ESSENTIAL AMINO ACIDS PRESENT IN VARIOUS PROTEINS

	casein	gelatin	zein	soybean	wheat flour
cystine	0.3	0.1	0.8	0.6	1.9
methionine	3.5	0.8	2.4	2.0	3.0
isoleucine	6.5	1.7	4.3	4.7	3.7
leucine	12.5	3.7	23.7	6.6	12.0
lysine	6.9	4.3	0.0	5.4	1.9
phenylalanine	5.2	2.0	6.4	5.7	5.5
threonine	3.9	1.5	2.4	4.0	2.7
tryptophane	1.8	0.0	0.1	1.6	0.8
valine	7.0	2.5	2.4	4.2	3.4

From these figures certain interesting observations can be made. For example, the excellence of milk as a protein food is attested by its casein content which with lactalbumin provides all the essential amino acids. Gelatin is not a complete protein since it lacks tryptophane and zein is incomplete since it lacks the essential component

lysine. The excellent supplementation offered by including soybean meal with foods poor in protein is indicated by its amino acid composition. Its inclusion in many food products greatly increases their nutritive value.

Certain normal conditions may result in greatly increased protein requirements so that during pregnancy and lactation the need for protein increases some 40-65 per cent. Persons on a low caloric diet require increased protein intake since the body is forced to derive its heat and energy requirements from protein due to the lack of carbohydrate. This results in a diminished supply of protein for other essential purposes.

Even though the protein intake may be normal, if it is improperly utilized and assimilated a protein deficiency may result. A number of abnormalities can cause the improper utilization of proteins ingested orally by an individual. First gastric achlorhydria, a condition commonly seen in pregnancy and anemia, impairs peptic digestion since the activity of pepsin is greatly diminished in the absence of sufficient hydrochloric acid. Patients with duodenal ulcer who are placed on antacids also have their gastric acidity and peptic activity reduced. A derangement of pancreatic function such as in pancreatic fibrosis even more seriously hinders protein digestion.

Intestinal obstruction prevents normal functioning, and diarrhea or colitis of long standing seriously interferes with normal assimilation by the intestinal mucosa which by reason of the inflammatory processes cannot normally assimiliate food products.

The third group of conditions which may result in protein deficiency is that wherein an excessive excretion or destruction of protein takes place. Chronic bleeding, which may be of intestinal origin and escape notice, excessive menstruation etc. places a drain on the blood regenerative processes which require amino acids. Nephritis with its increased permeability of the kidney tubules permits blood albumin to escape and results in hypoproteinemia. This may be a profound loss of body protein. Extensive burns cause serious consequences not only because of fluid loss but also because blood and tissue proteins are lost in the exudate. Inflammations, ulcers, abscesses, infections, and similar conditions are now known to produce a negative nitrogen balance and a demand for protein for purposes of reconstruction. Fevers, since they usually are accompanied by conditions making increased or even normal food consumption im-

possible, result in weight loss and a wasting of body tissues. This is due in part to a "raiding" of the tissues for essential amino acids needed to carry on basic functions that are of primary importance. Hyperthyroidism by producing a high basal metabolism often is accompanied by an excessive protein loss and negative nitrogen balance.

When one realizes that many conditions may result in two or even all three of these groups of conditions: insufficient intake, improper utilization and excessive loss, the clinical significance of amino acid therapy is obvious.

Determination of Protein Depletion or Insufficiency

In many cases the existence of protein deficiency is so clearly manifest that no clinical determinations are necessary to establish what is so apparent. In less well-defined cases blood determinations of the serum albumin/globulin ratio is made. Evidence of protein deficiency appears in this ratio inasmuch as protein insufficiency is coped with by the body by breaking down the less essential proteins such as serum albumin in order to obtain the amino acids needed to maintain essential organs. Serum globulin remains fairly constant even in protein depletion so the A/G ratio (albumin/globulin) is determined.

The exact quantitative relationship between hypoproteinemia and tissue protein depletion is not definitely known although experiments would indicate a 30:1 ratio between tissue protein loss and total plasma albumin loss. Total plasma protein determinations are not fully satisfactory for the detection of protein deficiency for two reasons. First, in many infections the fall in albumin is accompanied by a rise in globulin and, second, a fall in total circulating plasma proteins is often accompanied by dehydration with consequent hemoconcentration.

There seems to be little doubt that protein deficiency states are much more prevalent than has been considered likely heretofore. In fact the correction of this common condition with its often serious consequences has greatly improved the prognosis of many illnesses, surgical cases and injuries.

Clinical States Wherein Amino Acid Therapy Is Indicated

In the relatively short time that amino acid therapy has been tried a large number of clinical conditions have been found to respond to

its proper application. In many cases recovery or improvement has been so pronounced that physicians everywhere now accept such treatment as of primary importance in caring for their patients. The following conditions are now considered as indications for amino acid therapy:

Nutritional Edema—Whether it be known as hunger swelling, war edema, alimentary dropsy or prison edema, the condition is identical. It may be a primary condition or a secondary one brought on by other chronic diseases. In all cases swelling, particularly of the extremities, takes place as a result of the lowered colloid osmotic pressure of the blood due to hypoproteinemia. Following the war many persons in Europe were found suffering from starvation to the point that this form of edema had occurred.

In many cases the condition was so advanced that oral feeding could not be instituted since it caused vomiting. Intravenous amino acid therapy made possible the saving of thousands of lives with those who would have been without it hopeless cases. Nutritional edema can also be produced where impaired assimilation or utilization depletes blood protein even though the dietary intake seems adequate. Such cases are more likely to be seen by the clinician than frank cases of starvation.

Anemia—The role of iron and the erythrocyte maturing factor in the production of red cells and hemoglobin is well known. It should be remembered however that the so-called extrinsic factor which is of dietary origin is doubtlessly protein in character. Furthermore, the hemoglobin is deposited within the erythrocyte in a stroma for which adequate protein is needed. Faulty protein metabolism leads to a weakening of this stroma with an accompanying cell fragility and as a result rapid rate of cell destruction. It is well known that anemia is often accompanied by gastric achlorhydria, anorexia and a distaste for meat so all these factors favor protein depletion.

Pregnancy and Lactation—The protein concentration of the blood gradually falls during the first six months of pregnancy and the drain placed upon protein metabolism during lactation is readily perceived. It is believed that a high incidence of protein deficiency exists among pregnant women and the National Research Council recommends a 40 per cent increase in protein intake during the latter half of pregnancy and a 67 per cent increase during lactation.

The achlorhydria frequently associated with pregnancy is a condition that may result in protein insufficiency through faulty digestion and it has been well established that the all-important calcium assimilation is hindered if insufficient protein is consumed. For these reasons amino acid therapy is frequently indicated during pregnancy and lactation where the increased need for protein is or can not be met by dietary increase.

Pediatrics—Undernourished children may be the result of improper dietary habits such as excessive carbohydrate consumption which satisfies the energy requirements and causes loss of appetite. Whatever the cause of anorexia, a diet poor in protein leads to underweight, lowered resistance to infection and many other conditions unfavorable to normal growth and development. Many such cases require amino acid therapy as well as correction of diet to overcome the condition satisfactorily.

Liver Disease—High protein diet and amino acid therapy have been found greatly to improve various pathologic conditions of the liver such as cirrhosis and fatty degeneration. One of the essential amino acids, methionine, is now available and recommended almost as a specific in certain toxic conditions of the liver such as carbon tetrachloride or chloroform poisoning. Since an impaired liver leads to many other disturbances in the body this therapy is of utmost importance.

Kidney Disease—Kidney disease is often associated with increased permeability of the epithelium lining the tubules. The result is loss of blood protein, principally albumin with albuminuria and hypoproteinemia.

The old practice of restricting dietary protein in such cases is now considered quite inappropriate since more protein is indicated to compensate for the excessive loss which may run as high as 10-20 Gm. a day in the urine. Except in uremic poisoning, amino acid therapy in kidney diseases has proven quite helpful both in improving the prognosis and correcting the hypoproteinemia associated with the condition.

Burns—Extensive burns are serious on three counts: danger of infection, fluid loss and loss of protein. The first of these has been reduced by the sulfonamides and penicillin, and fluid can be given

readily by transfusion. Much of the almost miraculous recoveries from extensive burns is due to the early use of plasma as an immediate step to correct loss of blood protein and combat shock, followed by amino acid therapy which after the initial emergency may be substituted for plasma, permitting the body to regenerate its own protein. The healing rate of burns is remarkable under such therapy and it is now widely employed.

Ulcers—The healing of ulcers both duodenal and dermatologic is accomplished by amino acid therapy in an almost miraculous manner. The essential building blocks for tissue repair being thus supplied, healing rates are greatly increased. Many chronic cases have responded to this therapy which were incompletely healed or unaffected by other forms of treatment. Thus varicose, diabetic and decubitus ulcers are indications for amino acid therapy. Dr. Co Tui of New York University School of Medicine has carried out extensive studies of amino acid therapy in duodenal ulcers and his results were very striking. The rationale of amino acid therapy in this condition is interesting. Persons suffering from gastric ulcer are usually on a restricted diet with added alkalies to neutralize gastric acidity. This inactivates the pepsin so that protein digestion is impaired. It is considered quite probable, according to recent experiments, that low protein diets may actually induce ulcers. With protein depletion healing of the ulcer is made most difficult if not impossible. Amino acid therapy on the other hand neutralizes gastric acidity since the amino acids are ampholytes and bind free acids. Protein deficiency cannot result for the amino acids do not require digestion prior to assimilation. The result is acid neutralization without impairing the protein supply for the body and a rapid healing of the ulcer.

Surgery—No indication for amino acid therapy is more frequent than in the pre-surgical conditioning of a patient and in shortening convalescence. Many patients requiring surgery are in poor physical condition as a result of some chronic condition, anxiety leading to anorexia or other reason. It is a well-established fact that both anesthesia and surgery result in a very high loss of body nitrogen excreted in the urine. A patient already having or bordering on hypoproteinemia is in no condition to withstand the trauma of an operation with its attending nitrogen loss. The use of amino acid therapy to prepare a patient for an operation is now favored where time permits.

The period of convalescence following either surgery or prolonged illness involves a patient who either has no appetite or is unable to assimilate food properly. Tissue repair, healing, and gain in weight all require adequate protein. Amino acid therapy since it does not require digestion provides the answer. Rapid convalescence has been demonstrated by such therapy in many cases that otherwise would be quite prolonged. It is quite likely that such therapy in convalescence may become standard practice.

Other Indications—A number of other indications are established for amino acid therapy, such as in preventing protein insufficiency during the institution of elimination diets in tracing food allergies and in increasing resistance to infection. In the case of food allergy it is significant that amino acids and protein hydrolysates are non-allergenic so that a casein digest (hydrolysate) causes no reaction even in an individual sensitive to casein.

Although the eight amino acids known to be essential for man will, if given in adequate amounts, provide the necessary building blocks for a well-balanced protein metabolism, such a regimen is impractical for a number of reasons. The synthesis of amino acids is usually difficult and the cost of pure amino acids makes their routine use too expensive. Then, too, the synthesis of amino acids forms an equimolecular mixture of the optical isomers and it has been found that in some instances the unnatural isomers are either improperly utilized or they may even be potentially harmful when administered. These considerations have led to the preparation of hydrolysates of certain proteins known to be complete and to yield upon hydrolysis adequate amounts of all the essential amino acids.

The proteins most commonly employed for the preparation of hydrolysates are casein and lactalbumin, although other materials such as beef and cereal proteins are also used.

The hydrolysis is a carefully controlled process catalyzed by either acid or by pancreatic enzymes. In the case of acid hydrolysis a more complete break-down of the protein into amino acids occurs but the essential amino acid tryptophane is destroyed. It is necessary to add tryptophane of synthetic origin to such hydrolysates in order to repair this loss. Pancreatic digestion of the protein does not destroy the tryptophane but it is not as complete and an appreciable amount of peptids remain in the finished product. The presence of such peptids is not necessarily disadvantageous since it seems

well established that these small protein segments can be utilized by the body in most cases as well as amino acids themselves. In fact some workers claim their presence as a distinct advantage.

Most commercial hydrolysates also contain carbohydrate which, as has been explained, provides a sparing effect on the amino acids. In depleted states unless the carbohydrate needed by the body for its energy requirements is available the amino acids are utilized for this purpose rather than for protein synthesis. The presence of carbohydrate (dextrose) in products intended for parenteral administration has also been found highly advantageous in reducing the side reactions accompanying intravenous injection of the hydrolysate.

Products intended for oral use are frequently the cause of objection by the patient because of their unpleasant taste. In such cases mixing with some fruit juice, tomato juice or beverage will be found helpful. By changing frequently the vehicle used in administration much of objection on the part of the patient can be overcome.

When given parenterally it is important that the rate of administration be slow since if given too rapidly nausea and flushing may occur. Rapid administration is also disadvantageous inasmuch as the kidney threshold when exceeded results in loss of the amino acids by urinary excretion. It is recommended that, using a 5 per cent solution of a protein hydrolysate, not less than 2 hours be allowed for the infusion of each liter.

Hydrolysate Products

Amigen Powder—Mead Johnson & Company.

A casein hydrolysate made by pancreatic hydrolysis. For oral use or in solution by gastric or duodenal tube. It is readily soluble in water and for oral use it may be dissolved in carbonated drinks or fruit juices.

Nutramigen—Mead Johnson & Company.

Contains Amigen powder 20%, olive oil 18%, Dextri-Maltose 42.3%, starch 10%, yeast 3%, calcium gluconate 3.5% and mineral salts 3.2%. For oral use or, in smooth aqueous suspension, by gastric or duodenal tube.

Amigen Solution 10%—Mead Johnson & Company.

A sterile, pyrogen-free solution intended for parenteral injection following appropriate dilution with saline or dextrose solutions.

Amigen Solution 5% with Dextrose 5%—Mead Johnson & Company.

A sterile, pyrogen-free solution for intravenous use.

Aminonat—National Drug Company.

A protein hydrolysate plus 2% added salt and 4% flavors. For oral use with water, milk, fruit juice or other suitable vehicle.

Aminovite—National Drug Company.

A powder containing protein hydrolysate 70%, autolyzed yeast 10%, Hi-Ribo 2%, gelatin 1%, lactalbumin 10%, liver powder and flavors. For oral use as above, supplying not only amino acids but the B complex factors from yeast and liver, and minerals.

Aminoids—Arlington Chemical Company.

A protein hydrolysate derived from beef, milk, wheat and yeast with added sugars. For oral use.

Lactamin—Wyeth, Incorporated.

A protein hydrolysate made from lactalbumin by pancreatic digestion. Claimed to be richer in methionine than most products. For oral use and, in solution, by gastric or duodenal tube.

Parenamine—Frederick Stearns and Company.

An acid casein hydrolysate fortified with *dl*-tryptophane. Supplied as a 15% solution for oral use mixed with fruit juices, etc. or for parenteral use diluted with 5% dextrose or normal saline.

Protein Hydrolysate—Walker.

An enzymatic digest of casein together with all the required vitamins, iron, calcium and phosphorus (as phosphate). For oral use in solution.

Other Products

Delcos Granules—Sharp & Dohme.

A protein concentrate made from casein and lactalbumin. This is not a hydrolysate but it is recommended where increased protein in the diet is indicated. Claimed to have the advantage of excellent palatability but digestion, of course, is required for it to be utilized.

Meonine—Wyeth, Incorporated.

Synthetic *dl*-methionine. Methionine, one of the essential amino acids, has other important therapeutic uses. It acts almost as a specific in the treatment of certain types of liver damage, e. g., that arising from carbon tetrachloride poisoning. It is now recommended for liver damage associated with nutritional deficiency, cirrhosis, arsenotherapy and as a prophylactic in anesthesia. Available as crystals and 0.5 Gm. tablets.

A NEUTRALIZING MEDIUM FOR EVALUATING THE GERMICIDAL POTENCY OF THE QUATERNARY AMMONIUM SALTS

By R. Quisno, Irvin W. Gibby and Milton J. Foter *

THE problem of evaluating anti-bacterial agents has been complicated by the necessity of distinguishing between bacteriostatic and bactericidal effects of the various germicides.

With the older less potent cresols and phenolic compounds simple dilution served as an effective and practical means of making this distinction. The amount of germicide transferred from the medication tube to the subculture broth was insufficient to exert any bacteriostatic effect. However, the dilution method becomes impractical with compounds which are bacteriostatic in high dilution. The transfer technique described by Leonard (1) is actually a dilution method and is inaccurate because the bacterial sample is diluted in the same manner as is the germicide. Thus the quaternary ammonium salts must be diluted more than 1:1,000,000 to overcome bacteriostatic effects. At such high dilutions uniform sampling of the bacterial suspension is very unlikely. The use of large volumes of media as recently described by Klarmann and Wright (2) is cumbersome, time consuming, and uneconomical.

The problem was solved satisfactorily for the mercurial germicides by Brewer (3) and Heinemann (4). These workers added a specific neutralizing agent, sodium thioglycollate, to the subculture broth. Flett (5) employed ferric chloride to neutralize any phenol present in subcultures. His results showed a considerable drop over controls made in F. D. A. medium. Thus if an antidote for phenol is applied in the subcultures, phenol is not nearly so efficient.

Formulation of a medium which would have an analogous effect upon the cationic germicides appeared to be the only effective and usable *in vitro* method for overcoming the powerful bacteriostatic effects of these compounds. Such a medium should be capable of neutralizing the largest amount of germicide which would be transferred into the subcultures under actual test conditions. The medium should be clear and should be easy to prepare. A medium which meets all these requirements is described in this report.

* Department of Bacteriology, Research Laboratories, The Wm. S. Merrell Company, Cincinnati, Ohio.

Sodium stearate, serum, sodium thioglycollate and various anionic detergents were tested for their suitability as neutralizing ingredients. All of these substances were rejected for one or another reason. Several of the substances caused an undesirable turbidity in the broth, some of the ingredients were bacteriostatic and some were ineffective as neutralizing agents. The routine use of sterile serum in subculture broth is cumbersome and impractical. Low dilutions of the cationic germicides are not completely neutralized by serum.

A high degree of neutralization and a clear easily prepared medium was obtained by the addition of lecithin [1] and Tween 80 [2] to standard F. D. A. broth [3] or any other good subculture medium. Lecithin acts as the chief neutralizer while the Tween acts as a solubilizing and dispersing agent for the lecithin.

The method of preparation and the formula for the medium are as follows:

Beef extract (Difco)	5.0 gm.
Peptone (Armour)	10.0 gm.
Sodium chloride	5.0 gm.
Lecithin	0.7 gm.
Tween 80	5.0 gm.
Distilled water	1000 ml.

The lecithin and Tween are added to approximately 400 ml. of hot water. This mixture is boiled briefly and allowed to stand while the other ingredients are dissolved in a separate container. The lecithin-Tween mixture is mixed with the medium ingredients and the combined broth is boiled for ten minutes, filtered thru coarse filter paper and made up to volume. The pH is adjusted to 6.8-7.0 and the medium is placed in tubes and sterilized. While hot the medium may appear cloudy, but it becomes clear upon cooling. For convenience the name "Letheen" has been used to describe this medium.

To determine the neutralizing capacity of Letheen broth, one ml. quantities of various dilutions of potent quaternary ammonium salts were added to ten ml. quantities of the medium as shown in the

[1] Lecithin obtained from American Lecithin Co., Inc.

[2] A polyoxyethylene derivative of sorbitan monooleate obtained from Atlas Powder Co.

[3] Circular No. 198, United States Department of Agriculture.

table. The tubes were inoculated immediately with 0.1 ml. of a 24 hour culture of *Staphylococcus aureus* and incubated at 37° C. for 48 hours. After incubation, growth was determined by the appearance of turbidity and by microscopic examination of the cultures. For comparison the same test was performed with F. D. A. broth. It should be noted that this is not a germicide test, but rather a test of the bacteriostatic effect of various concentrations of a cationic germicide in subculture media.

The results demonstrate conclusively that the Letheen medium rapidly overcame the bacteriostatic effect of relatively high concentrations of cationic germicides. Ten ml. of Letheen broth neutralized one ml. of a 1:1500 dilution of the germicide, while growth failed to occur in the F. D. A. medum in the presence of one ml. of a 1:50,000 dilution of the germicide.

Discussion

The formulation of an easily prepared medium which effectively neutralizes the quaternary ammonium salts makes possible an accurate examination of the true germicidal powers of these compounds. Bacteriostatic effect no longer complicates the picture.

THE EFFECT OF A NEUTRALIZING SUBCULTURE BROTH ON THE BACTERIOSTATIC POWER OF QUATERNARY AMMONIUM SALTS

Quantity of Germicide Added to Subculture Broth	Growth in Subcultures	
	Letheen	FDA Broth
1 ml of 1:1000 dilution	— +	— —
1 " of 1:1500 "	+ +	— —
1 " of 1:2000 "	+ +	— —
1 " of 1:5000 "	+ +	— —
1 " of 1:20,000 "	+ +	— —
1 " of 1:40,000 "	+ +	— —
1 " of 1:50,000 "	+ +	— —
1 " of 1:100,000 "	+ +	+ +
1 " of 1:120,000 "	+ +	+ +
1 " of 1:140,000 "	+ +	+ +

Test organism = *Staphylococcus aureus.*

A test period of ten minutes has been adopted as standard for routine *in vitro* evaluation of germicides. It is no doubt true that the antibacterial action of most germicides extends considerably beyond the treatment period if neutralizing subculture media are not used. To obtain precise information about the speed of reaction of germicides and to perform accurate deathtime studies, neutralizing subculture media are of primary importance.

It has long been known that negatively charged fatty acid ions were capable of neutralizing the germicidal properties of the positively charged cationic germicides. The phospholipid, lecithin, rendered soluble by the dispersing agent, Tween 80, is also a potent neutralizing agent.

Lecithin and Tween 80, in the concentrations given in the formula, do not inhibit the growth of organisms which will grow in F. D. A. broth. Some strains of streptococci which will not grow in unenriched F. D. A. broth grow abundantly in Letheen broth.

Summary

A subculture medium has been formulated which effectively neutralizes relatively high concentrations of quaternary ammonium salts. The medium is clear, easy to prepare, and readily reproducible.

The formulation of this medium permits accurate *in vitro* evaluation of the bactericidal properties of cationic germicides and eliminates bacteriostasis in the subculture.

REFERENCES

(1) Leonard, G. F.: Limitations of Phenol Coefficient Tests in Determining Germicidal Activities. *J. Infectious Diseases, 48,* 358-365 (1931).

(2) Klarmann, E. G., and Wright, E. S.: An Inquiry into the Germicidal Performance of Quaternary Ammonium Disinfectants. *Soap and Sanitary Chemicals, 22,* 125-137 (1946).

(3) Brewer, J. H.: Clear Liquid Mediums for the "Aerobic" Cultivation of Anaerobes. *J. A. M. A., 115,* 598-600 (1940).

(4) Heinemann, B.: Comparison of Methods Used for Evaluating the Bactericidal Properties of Mercurial Compounds. *Jour. Amer. Pharm. Assoc., 32,* 298-301 (1943).

(5) Flett, H. L., *et al.*: The Revival of Organisms Presumably Killed by Phenol. *J. Bact., 50,* 591-595 (1945).

A NEW PROCEDURE IN THE DETERMINATION OF ALCOHOL IN GALENICAL PREPARATIONS BY THE CUSTOMARY DISTILLATION METHOD II.*

By Sigmund Waldbott **

THE following procedure is a modification of the method given in *I** in order to approximate its details more nearly to usual pharmacopoeial practice.

The 100 cc. glass-stoppered flask is herein used solely for collecting the condensate obtained as before from 150 cc. of hydro-alcoholic liquid containing either 50 cc. or 25 cc. of the galenical preparation. As in *I*, distillate is collected *above the 100 cc. mark, i. e.* between 102 and 104 cc. and *its net weight is determined* (=*a*) without the need of noting the temperature.

In the following tables, on the other hand, we have been using for Alcohol determinations a *"thermopyknometer"*, 50 cc., of the well-known type, *i. e.* stoppered with a sensitive thermometer, and having a bent-up side tube with ground-edged cap.

The glass weights of the 100 cc. receiving flask and of the complete pyknometer are first determined; the tabulated results state *net* weights only.

Another preliminary determination is that of the *water-value* at 25° C. of the pyknometer for use in the calculation of *specific gravity*. With our pyknometer, the water-value is 49.7808 gm. per 50 cc., or 99.5616 gm. water per 100 cc. of receiving vessel. If, for example, *b* is the net weight of alcoholic distillate filling a 100 cc. vessel at 25° C., its *specific gravity* is *b*/99.5616.

It is better, however, to *multiply b* with the *reciprocal* of 99.5616=.010044; then *spec. gravity*=*b* × .010044. Calculations in the subsequent tables are thus greatly simplified.

In the following tables of results, the successive steps of the method are indicated by the consecutive headings at the top of the

---

* *Cf. I.* Paper read before the U. S. Pharmacopoeial Convention, Public Scientific Conferences, May 13, 1940, Washington, D. C., *Circulars* 24, p. 55-XX; *Chem. Abstracts*, 1940, 8181.

** Laboratory of Lloyd Brothers, Pharmacists, Inc., Cincinnati 2, Ohio.

columns. For example, in Column VI the weights of 100 cc. of distillate obtained at 25° C. are recorded; in Column VII their specific gravities, in Column VIII their intermediary Vol. % of Alcohol at 15.56° C.

To extend the weight of 100 cc. distillate to that of the total distillate, an obvious *extension factor* (a/b) is given in Column IX; this in Column X is multiplied by results in Column VIII, and by 2 or 4 to obtain final alcohol % by volume at 15.56° C., well comparable with the expected values in Column II.

I Galen. Prepns.	II Alcohol Vol. % expected	III Distil 150 cc. cont'g. 50 cc. or 25 cc.	IV a = net wt. of total distillate	V c = net wt. of distillate in 50 cc. pyknometer at 25° C. *Figures omitted* = half of Col. VI	VI $b = 2 \times c$ = wt. of 100 cc. distillate at 25° C.	VII Spec. grav. at 25° C. = $b \times .010044$	VIII Alcohol Vol. % see U. S. P. table 25°/25° C. p. 793	IX a/b is *extension factor*	X Alcohol final Vol. % calcd. = Col. VIII × Col. IX ×2 or ×4
Alcohol below 30%									
Special Mixture	15 %	50 cc.	101.1974		98.5252	.98958	7.39	1.027	15.17%
Chionanthus	15.7	50	100.797		98.4892	.9892	7.60	1.026	15.59
Nux Fer. Gen	18	50	100.7352		98.3432	.98776	8.8	1.024	18.02
Lloydrastis	21.7	50	100.005		98.1404	.9857	10.41	1.0189	21.21
Crataegus	25	50	100.3456		97.9564	.98387	11.95	1.024	24.47
Geranium	25.3	50	99.6198		97.9232	.9835	12.21	1.0173	24.84
Alcohol at or near 30%									
Fraxinus	30	50	100.068		97.6824	.98112	14.23	1.024	29.14
Capsella	30	50	99.769		97.6664	.98096	14.35	1.021	29.30
Lobelia	30	50	100.040		97.6456	.98075	14.54	1.032	29.77
Spigelia	30	50	99.2484		97.6292	.98058	14.67	1.016	29.80
Bryonia	30	50	100.3876		97.6504	.98.08	14.5	1.028	29.81
Arnica	30	50	99.2446		97.6268	.9805	14.69	1.016	29.85
Gentian	30	50	99.7452		97.6360	.98065	14.62	1.021	29.85
Red Clover (Trifolium)	30	50	99.9168		97.6376	.98067	14.60	1.023	29.87
Pinus Can.	30	50	100.6638		97.6484	.98078	14.51	1.030	29.89
Hydrangea	30	50	100.8699		97.6532	.98083	14.40	1.032	29.90
Matricaria	30	50	100.0326		97.6268	.98056	14.69	1.0245	30.08
Chimaphila	30	50	99.7198		97.6156	.98045	14.79	1.023	30.26
Chelidonium	30	50	100.0586		97.6180	.98047	14.77	1.025	30.27
Colocynth	30.8	50	99.9632		97.6172	.9804	14.85	1.024	30.37
Lycopus	30	50	99.6606		97.5966	.98029	14.92	1.021	30.46
Sambucus	30	50	100.2581		97.5938	.98028	14.97	1.027	30.57
Rumex	30	50	100.1439		97.5972	.98024	14.96	1.026	30.69

Valerian	30	50	100.3630	97.5972	.98026	14.90	1.028	30.72
Euphrasia	30	50	100.1471	97.5876	.98016	15.00	1.026	30.78
Aralia	30	50	100.1092	97.5636	97993	15.22	1.026	31.23
Euphorbia	30.9	50	99.9106	97.5500	.9798	15.33	1.024	31.39
Taraxacum	30	50	99.6206	97.5258	.9795	15.54	1.021	31.43
Scutellaria	30	50	99.9486	97.5344	.97963	15.47	1.0247	31.69
Agrimonia	30	50	99.6362	97.5084	.9794	15.33	1.021	31.97
Amygdalus	33.2	50	99.9366	97.4652	.9789	16.08	1.023	32.96
Alcohol above 30% to 70%								
Ambrosia	32.1%	50	99.5194	97.4574	.9788	16.01	1.021	32.7%
Stillingia	38	25	101.0414	98.2784	.9871	9.30	1.028	38.24
Phytolacca	38	25	99.8772	98.2384	.9867	9.92	1.016	39.70
Spongia	45	25	100.0720	98.0772	.98509	10.91	1.020	44.53
Collinsonia	46	25	100.6250	98.0368	.98468	11.26	1.026	46.21
Berberis	50	25	101.0010	97.9152	.98346	12.28	1.031	50.64
Dioscorea	50	25	100.4210	97.8924	.9832	12.5	1.025	51.25
Grindelia	63.6	25	99.8688	97.5500	.9798	15.33	1.023	62.73
Echinacea	65	25	99.4243	97.4748	.97903	15.97	1.020	65.15
Aesculus (Horsechstnt.)	65	25	99.4886	97.4520	.9788	16.16	1.021	65.99
Eryngium	68	25	99.6456	97.3876	.97816	16.7	1.023	68.33
Macrotys	70	25	99.5110	97.3288	.97757	17.2	1.022	70.31
Gossypium	70	25	99.1880	97.3156	.9774	17.33	1.019	70.63
Alcohol above 70% to 94%								
Asclepias	72	25	99.8810	97.2872	.97715	17.59	1.026	72.18
Podophyllum	71	25	100.7256	97.2752	.9770	17.69	1.035	73.25
Saw Palmetto	75	25	99.4126	97.1964	.97624	18.45	1.022	75.42
Leptandra	75	25	99.5382	97.1920	.9762	18.45	1.025	75.64
Cactus	77	25	99.4070	97.1374	.97565	18.95	1.023	77.54
Rhus Toxicodendron	78	25	98.9279	97.1120	.97538	19.18	1.018	78.10
Pulsatilla	78	25	99.4668	97.0936	.9752	19.32	1.024	79.13
Iris	80	25	99.0796	97.0256	.97452	19.92	1.021	81.35
Thuja	82	25	98.8646	96.9752	.97402	20.38	1.019	83.06
Piper Methysticum	85	25	99.3738	96.9602	.9738	20.48	1.024	83.87
Sticta	85	25	99.5564	96.9076	.97334	20.96	1.027	86.10
Elaterium	94;							
Elaterium	94.72	25	98.8206	96.6700	.9708	23.16	1.022	94.67

The publication of the foregoing material has been approved by the management of Lloyd Brothers, Pharmacists, Inc.

SELECTED ABSTRACTS

Some Aspects of Penicillin Stability. E. L. Smith. *Pharm. J. 103,* 71 (1946). Although pure sodium penicillin was found to be unchanged after several hours of heating at 100° C., buffered aqueous solutions of pure penicillin II were much less stable. Pure penicillin in dilute solution at pH 6.0 has a half-life at 24° C. of 14 days. The rate of destruction is doubled at pH 5.3 or 7.5; it is 10 times as great at pH 4.5 or 9.0, and 100 times as great at pH 3.2 or 10.5. The rate of inactivation increases about threefold for each 10° C. rise in temperature.

Heavy metals such as copper, zinc, lead and mercury, and to a lesser degree, iron, appear to exert a catalytic effect in destroying penicillin. As little as 0.2 p. p. m. of copper and 2 p. p. m of zinc can inactivate penicillin in the absence of oxygen or oxidizing agents, although exposure to air or low concentrations of hydrogen peroxide accelerates the destruction Since organic peroxides inactivate crude penicillin, solvents used in its extraction or in analytical procedures should be peroxide-free. The use of peroxidized oils should be avoided when preparing oily suspensions or ointments of penicillin.

Tests of several antoxidants and reducing agents as stabilizers for penicillin failed to yield satisfactory results. More satisfactory results were obtained with certain substances known to form complexes with heavy metals, *e. g.,* sodium hexametaphosphate (Calgon), of which 0.1 per cent produced a marked stabilizing effect on solutions of either commercial or pure crystalline penicillin.

Pure penicillin in 70 per cent acetone (150 I. U./mil), stabilized with Calgon, showed an insignificant loss on storage for three months at 24° C. and about 15 per cent loss at 37° C. It is suggested that such solutions may be useful for the treatment of small wounds or for pre-operative skin sterilization.

It was observed that concentrated solutions of penicillin appeared to deteriorate faster than weaker ones.

Lactic acid was found to cause 70 per cent inactivation of penicillin in one day at 24° C., when used in a weight equal to that of the antibiotic. *p*-Aminobenzoic acid had a slight inactivating effect, but butyl alcohol (5 per cent) phenylacetic acid and β-alanine had no significant action.

A Note on Khellinin as a Coronary Dilator. K. Samaan. *Quart. J. Pharm. & Pharmacol. 19,* 135 (1946). The method of isolation and a description of the properties of khellinin ($C_{19}H_{20}O_{10}.2H_2O$), a glycoside of Ammi Visnaga, were published by the author in 1931 and 1932.

Pharmacological investigations on rabbits revealed that khellinin in concentration, as low as 1 :100,000 increased the contractility of the cardiac muscle by producing a more complete systole and a more complete diastole with a corresponding increase in cardiac output. It increased the coronary flow of the isolated heart, and it raised the blood pressure in the intact animal.

If partial experimental spasm of the coronary vessels was first induced by the use of barium chloride solution (1 :40,000), the degree of coronary dilatation produced by khellinin in concentrations of 1 :25,000 to 1 :300,000 is more pronounced.

This glycoside is stated to be a drug of very low toxicity. Its M. L. D. by intralymphatic injection was found to be 1 gm./Kg. of body weight of toad in 24 hours.

Efficacy of Penicillin Ointments. J. A. Heinlein, C. C. Carpenter, A. Yaguda and J. C. Wyse. *U. S. Naval Med. Bull. 46,* 1237 (1946). The relative effectiveness and stability of ointments containing penicillin incorporated in lanolin and in an oil/water emulsion base was studied *in vitro.* Filter-paper disks 1 cm. in diameter were impregnated with equal amounts of the ointments containing 400 units of penicillin per gram. The disks were placed on blood agar plates previously streaked with *Staphylococcus aureus,* and the zones of inhibition were measured after 24 hours.

Both types of ointments deteriorated markedly during storage at room temperature for about two weeks; no antibacterial activity was apparent after twenty-six days. When the ointments were refrigerated at 8° C., some activity was still possessed by the lanolin preparation after six weeks though the O/W ointment exhibited no potency after about one month.

Clinical trials of these ointments in cases of nummular eczema revealed that penicillin in the O/W emulsion base was more rapidly effective than when used in a lanolin base.

The Nature of the Antibacterial Agent From Anemone Pulsatilla. H. Baer, M. Holden and B. C. Seegal. *J. Biol. Chem. 162,* 65 (1946); through *U. S. Naval Med. Bull. 46,* 1206 (1946). Protoanemonin was isolated from the dried and ground plant *Anemone pulsatilla.* It was found to possess anti-bacterial activity against a wide variety of micro-organisms, including gram-positive, gram-negative, and acid-fast bacteria.

Protoanemonin appears to be a member of a group of antibacterial agents such as penicillic acid, clavacin and crepin which contain a five-membered unsaturated lactone ring and also a highly reactive system of double bonds. Hydrolysis of the lactone ring with alkali results in the loss of antibacterial power.

Protoanemonin rapidly polymerized to anemonin and higher polymers in the pure state.

Mechanism of Spontaneous Cure in Puberty of Ringworm of the Scalp. S. Rothman, A. M. Smiljanic and A. W. Weitkamp. *Science 104,* 201 (1946). Ringworm of the scalp hair caused by the fungus *Microsporon audouini* is endemic among school children in numerous large cities. Although the infection is persistent during early childhood, it is not seen in adults. The investigation was undertaken in order to ascertain the local changes on the scalp following puberty and the reason for the nonsusceptibility of the scalps of adults to *M. audouini* infections.

The ether extraction of 45 Kg. of adult hair yielded 230 gm. of free fatty acids, together with several kilos of neutral substances. The total ether extract of adult hair was found to be five times as effective as that of children in inhibiting the growth of *M. audouini* on culture media. A concentration of hair fat ten times greater was found necessary to inhibit the growth of such other pathogenic fungi as *Trichophyton gypseum, Achorion schoenleinii* and *M. lanosum,* to which the hair of adults is not immune.

The fungistatic activity of hair fat was found to reside in the portion of the free fatty acids which was volatile with steam, *i. e.,* a mixture of acids below C_{12}. Fractional distillation at 2 mm. pressure of the methyl esters of the mixed acids yielded 1 per cent of a

fraction containing C_7 to C_{11} acids. Pelargonic acid (a C_9 normal aliphatic acid) was prominent in this mixture; not all of the other components were identified. It is believed that human hair fat is unique among fatty materials, in that it contains a whole series of monobasic normal aliphatic acids having odd numbers of carbon atoms.

Therapeutic trials of the total extract, low-boiling fractions, pelargonic acid and its sodium salt, and sodium undecylenate, applied in a variety of ways, failed to exert a curative effect upon ringworm of the scalp in children. The cause of the negative results was found by *in vitro* experimentation to be the inability of either the free fatty acids or their sodium salts to penetrate the keratin material of the hair and thus reach the spores within it. Trials of mild keratolytic agents like sodium thioglycolate in combination with the fungicidal drug failed to yield encouraging results.

The local application of pelargonic acid or other fungicidal agent was found to be of value in preventing the spread of infection from a ringworm lesion to uninfected hairs.

Urinary Recovery of Penicillin After Oral Administration With Antacids and Buffers. M. J. Golden and F. M. Neumeier. *Science 104,* 102 (1946). The urinary excretion of penicillin was found to be greater when the drug was administered orally in combination with an antacid powder composed of sodium bicarbonate, colloidal kaolin, magnesium trisilicate and bismuth subcarbonate, or with sodium bicarbonate alone, than was the case when sodium citrate, aluminum hydroxide gel, dibasic sodium phosphate, calcium carbonate or cottonseed oil were used. Penicillin in normal saline solution was given orally as a control.

A single normal male test subject was used throughout the entire series. The dose of sodium penicillin was 25,000 units, dissolved in an aqueous solution or suspension of the antacid or buffer under test, and taken about two hours after breakfast. Urine samples were collected and measured after one-half hour and thereafter at hourly intervals for six hours. The penicillin titer was deter-

mined by the F. D. A. cylinder-plate method against *Staphylococcus aureus* 209, comparing the results with a standard penicillin curve prepared daily.

It was found impossible to obtain reproducible characteristic results on the urinary excretion of penicillin when females were used as the test subjects, an observation which has been reported by several investigators.

The Rapid Detection of Barbiturates in Urine. X. Denis and A. Lambrechts. *Rev. Belg. Sci. Med. 8,* 247 (1940); through *Quart. J. Pharm. & Pharmacol. 19,* 199 (1946). The method of Griffon and Breton for the determination of barbiturates in urine is recommended for the rapid confirmation of suspected poisoning by drugs of this group.

The urine is absorbed by anhydrous sodium sulfate, the latter is then packed in a tube over a layer of vegetable charcoal and a layer of magnesium oxide, and the barbiturate is extracted by percolation with ether. The percolate is collected in a warmed receiver in order to evaporate the solvent spontaneously. The presence of a barbiturate in the residue is detected by the use of Parri's reagent, cobalt nitrate and diethylamine in alcohol solutions. The entire procedure can be performed in an hour.

It is stated that the presence of phenobarbital can be demonstrated in the urine of a person who has received 0.2 gm. of the drug. The authors report that this method is sensitive to 0.2 mg. in 20 ml. of urine.

The Effect of Type of Capillary Glass on the Melting Point of Acetylsalicylic Acid. H. W. Johnson and C. W. Ballard. *Pharm. J. 103,* 88 (1946). The authors reported to the British Pharmaceutical Conference that discrepancies in the melting point of acetylsalicylic acid had been found by independent observers who followed the official procedure. Consultation of the literature revealed values ranging from 132° to 138° C.

The discrepancy was found to be independent of possible variations in interpretation of directions regarding the use of a capillary tube "about one mm. in diameter" and of the manner of heating, e. g., "about 5° below the expected melting point" and "about 3° per minute". Standardization of these factors and also of particle size between the author's laboratory and another which participated in the investigation failed to account for the variation.

It was demonstrated that the type of glass used in making capillary tubes affects the melting point of acetylsalicylic acid. The authors recommend that Pyrex type glass should be specified for melting point tubes, pointing out that the work of Jones and Wood (1941) indicates that sharper and more reproducible values can be obtained in this way.

The authors propose to extend this investigation to other pharmaceutical chemicals which exhibit a depression of the melting point when soft glass capillary tubes are used, in order to determine whether a revision of the official data is desirable.

Some Uses of Thrombin in the Surgery of the Ear, Nose, and Throat. P. Reading. *Brit. Med. J.* No. 4469, 295 (1946). Thrombin was first prepared by Mellanby in 1933; on addition to blood or plasma it causes the formation of a clot. The product used in the series of cases forming the basis of this report was Thrombin, Topical, P. D., supplied as a powder packaged in ampuls, each of which contains 5,000 Iowa units. This unit is defined as the amount of thrombin which will clot 1 ml. of standard fibrinogen solution in 15 seconds.

Trial of thrombin in 166 tonsillectomies by spraying the raw surfaces with its solution after the larger vessels had been tied was disappointing. It failed to prevent the reactionary hemorrhage occasionally encountered.

It was found that simple nose-bleed is not easily controlled by the application of thrombin. In all except 2 cases of 35 in which submucous resection of the nasal septum was performed, however, the use of thrombin eliminated the necessity of packing with gauze.

A technic of fixation of skin grafts by means of a fibrin clot following mastoidectomy is described. The precipitation of fibrin between the graft and its bed can be accomplished by the addition of thrombin to plasma. In 47 mastoidectomies in which this procedure was used, primary grafting was performed in 38 cases and delayed in 9. In 29 cases there was apparently complete survival of the graft; in 14, over 75 per cent of the graft survived; and in 4 the graft failed to "take".

Penicillin in Treatment of Common External Eye Infections. J. Minton. *Brit. Med. J.* No. 4470, 324 (1946). Excellent clinical results were obtained in the treatment of external eye infections in children through the use of penicillin eye drops and ointment. Allergic skin reactions were seen in a number of adults who received this therapy, but were absent in the children. The reaction consisted of a redness and edema of the skin of the lids, often extending to the skin of the cheek and forehead. The condition cleared up in a few days following discontinuance of the treatment.

The eye drops used contained 1,000 or 2,000 units of penicillin sodium per ml. and were prepared by adding the required number of tablets (B. & W.) of the drug to 20 ml. of sterilized distilled water. The pH of the solution is stated to be between 6 and 6.5 and to be non-irritating to the eyes. The pH of tears is 7.2. Biological assay of the preparation demonstrated that when it was stored at room temperature, and the bottles were uncapped frequently and exposed to the air, it retained 80 per cent of the original potency at the end of 14 days.

The penicillin ointment for ophthalmic use was prepared by triturating penicillin in powder form with liquid paraffin, and gradually incorporating eucerin L. M.; the strength was 400 units per gram. Aseptic technic was used throughout the process. Eucerin L. M. was found to be preferable to "Lanette" wax as a base, since the acidity of the latter caused the ointment to be irritating to the eyes. The pH of the ointment was 7.1, and did not change significantly on storage. No biological assay was performed on the ointment, but it is believed that the preparation remains stable for over six weeks.

New ZEPHIRAN*

CHLORIDE

TINTED SOLUTION 1:1000

ZEPHIRAN was widely used by the Armed Forces during the war period. Prior to that time and consistently since, this effective antiseptic enjoyed acceptance by the civilian medical profession and hospitals.

Therefore, it is a logical step to offer Zephiran Chloride Tinted Solution for everyday first aid treatment of cuts and superficial wounds. The handy applicator bottle in an attractive counter display carton will prove readily salable.

This new product is introduced under Fair Trade effective April 1

Efficient antiseptic

For First Aid Treatment of cuts and superficial wounds

No sting · No burn · Nontoxic

ZEPHIRAN CHLORIDE TINTED SOLUTION	M. F. T. Price
(1:1000 — ½ fluidounce) per bottle	$0.23

A liberal bonus goods offer is presented commencing May 1

Consult your wholesaler's salesman or Winthrop representative for details.

Supplied in attractive counter display carton of 24 convenient applicator bottles of ½ fluid ounce.

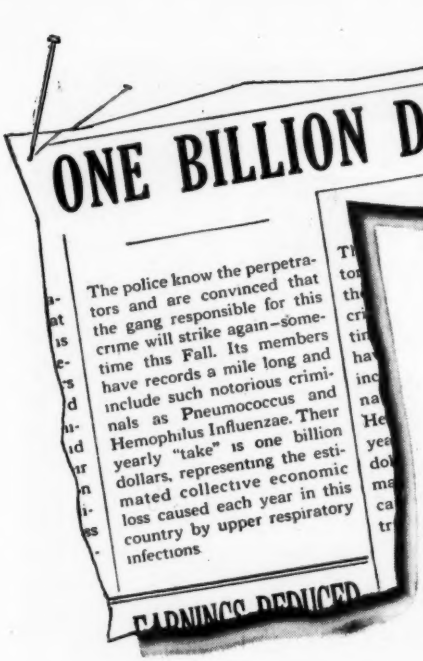
Complete WINTHROP CATALOG published in 1945-1946 American Druggist Blue Book and Drug Topics Red Book

*Trademark Reg. U. S. Pat. Off., brand of benzalkonium chloride (refined)

Winthrop **CHEMICAL COMPANY, INC.**

PHARMACEUTICALS OF MERIT FOR THE PHYSICIAN

170 VARICK STREET NEW YORK 13, N. Y.


ONE BILLION DOLLARS STOLEN
The police know the perpetrators and are convinced that the gang responsible for this crime will strike again—sometime this Fall. Its members have records a mile long and include such notorious criminals as Pneumococcus and Hemophilus Influenzae. Their yearly "take" is one billion dollars, representing the estimated collective economic loss caused each year in this country by upper respiratory infections

As leaders in the campaign to suppress and to prevent these infections, ORAL VACCINES are gaining more and more prominence. Outstanding among them is IMMUNOVAC.

IMMUNOVAC is different in that it offers not merely the immunizing properties of whole killed bacteria —the usual endo-antigens—but *in addition,* the soluble, surface ecto-antigens. This combination, made possible by a distinctive PARKE-DAVIS developed process, provides for *increased antigenic potency* without an increase in reactive properties.

Physicians favor initiation of the course of treatment *during the Fall* and continuation of a maintenance dose throughout the period of the year when respiratory infections are most frequently encountered.

THE SEASON OF HIGHEST DEMAND FOR IMMUNOVAC ORAL VACCINE IS ABOUT TO START and should find you prepared. We suggest you check your stock and order an adequate supply.

IMMUNOVAC *Oral Vaccine Tablets*

enteric coated, are available in bottles of 20, 100 and 500.

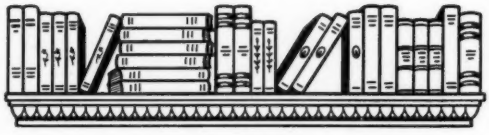
PARKE, DAVIS & COMPANY · Detroit 32, Mich.

USO
Keep it up...
Don't let them down!

Give
Now
To Your Community Chest

Ten

POPULAR SCIENCE VOLUMES



For reference or for interesting reading, the more-than-a-hundred topics included in the ten paper-bound volumes of Popular Science Talks are worthy of your consideration. Their value is far beyond the purchase price of $1.00 each, postpaid.

Send now for descriptive folder.

American Journal of Pharmacy

43rd Street, Kingsessing and Woodland Avenues

PHILADELPHIA 4, PENNSYLVANIA